described in the previous section. Radioactive leucine (5.0 mg.) and D-glucose (100 mg.) were dissolved in a few drops of 0.1 *M* acetate buffer, *p*H 5, in a small tube, which was then heated at approximately 80° for one hour in a water-bath. The semi-dry mixture, *still completely colorless*, was dissolved in 5.0 ml. 1.5 *N* hydrochloric acid and chromatographed on a cold Dowex-50 ion-exchange resin column. The result is shown in Fig. 2. The pattern is very similar to that obtained with liver extracts (Fig. 1). The radioactivity in the new compound amounted to 14% of radioactivity added as leucine, and only about 50% of the total radioactivity was recovered. Paper chromatographic analysis gave results similar to those already described for the compound isolated from liver extracts.

The Reaction between D-Ribose and Radioactive Leucine.—A mixture of 100 mg. of D-ribose and 5.0 mg. carboxyl-C^{14}-L-leucine were heated at *p*H 5 in a "semi-dry" condition as described for the glucose–leucine mixture. A great deal of browning took place, in contrast to the glucose–leucine reaction mixture. The chromatographic analysis is shown in Fig. 3. It should be noted that only a small amount, if any, free leucine remained. The brown colored products emerged at the "front" and then gradually diminished. A number of well defined radioactive peaks appeared but these products have not been analyzed further. The greater reactivity of ribose as compared to glucose is in accord with the observation of other investigators.[22]

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Absorption Spectra of Fuming Sulfuric Acid Chromogens Obtained from the Estrogens and Other Steroid Compounds[1]

BY LEONARD R. AXELROD

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Concentrated sulfuric acid has been observed to form chromogens with steroids which give absorption spectra different for each compound.[2] These absorption spectra have been utilized to aid in the qualitative identification of steroid metabolites.[3,4] Fuming sulfuric acid has now been found to form chromogens with the estrogens and other steroids which give specific absorption spectra for each compound different from those with concentrated sulfuric acid. The procedure is as follows: Three ml. of reagent grade fuming sulfuric acid (assay: 15–16% free SO_3) is added to 30–50 micrograms of steroid in a glass-stoppered test-tube. After one-half hour in the dark at room temperature, the optical density of the solution from 220–600 mμ is read in a Beckman D. W. spectrophotometer. Quartz cells with ground glass stoppers obtained from Pyrocell Co., New York, were utilized to protect the apparatus from the acid. Fuming sulfuric acid was used as a blank.

Table I summarizes the results obtained with 22 of the steroids studied. The shapes and peaks of the absorption spectra were found to be specific for each compound.

(1) This investigation was supported by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

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(4) A. Zaffaroni and R. Burton, *J. Biol. Chem.*, **193**, 749 (1951).

TABLE I

Compounds[5]	Absorption maxima
Estriol	430
Estradiol-17β	300, 430
Estradiol-17α	300, 420
7-Ketoestrone	242, 310, 425
Equilenin	310, 380, 445
Equilin	305, 380, 435
Δ^6-Dehydroestrone	300, 365, 435
Estrone	295, 380
Methoxydoisynolic acid	265, 320, 390
Diethylstilbestrol	425
17α-Ethinylestradiol	No maxima
17-Hydroxycorticosterone	240, 500
17-Hydroxy-11-dehydrocorticosterone	295, 440
17-Hydroxy-11-desoxycorticosterone	240, 275, 505
Corticosterone	240, 275, 410, 485
11-Desoxycorticosterone	240, 280, 490
Dehydroepiandrosterone	300, 405
Epiandrosterone	235, 300, 395
Testosterone	300
Androsterone	295, 390
Progesterone	300, 440
Pregnane-3α,20α-diol	285

It was furthermore found that the absorption spectra of most compounds change with time so that a new spectrum evolves if the chromogen solution is allowed to stand at room temperature for longer periods of time. For example, 17-hydroxy-11-dehydrocorticosterone after 24 hours exhibits maxima at 250, 280 and 495 mμ. This phenomenon has proven most useful for obtaining the qualitative identification of a single sample of steroid compounds over a period of 24 hours.

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A Triazolopyrimidine Analog of 6-Mercaptopurine[1,2]

BY CARL TABB BAHNER, BILL STUMP AND MARY EMMA BROWN

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The compound 6-mercaptopurine has been shown to inhibit growth of certain bacteria[3] and tumors.[4–6] Roblin, Lampen, English, Cole and Vaughn[7] prepared several triazolopyrimidines which were found to inhibit bacterial growth and one of them, 8-azaguanine, was found to inhibit certain tumors.

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(2) Presented in part at the Southeastern Regional Meeting of the American Chemical Society, Auburn, Alabama, October 24, 1952.

(3) G. B. Elion, G. H. Hitchings and Henry Vanderwerf, *J. Biol. Chem.*, **192**, 505 (1951).

(4) D. A. Clarke, F. S. Philips, S. S. Sternberg, C. C. Stock and G. B. Elion, *Proc. Am. Assn. for Cancer Res.*, **1**, 9 (1953).

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